

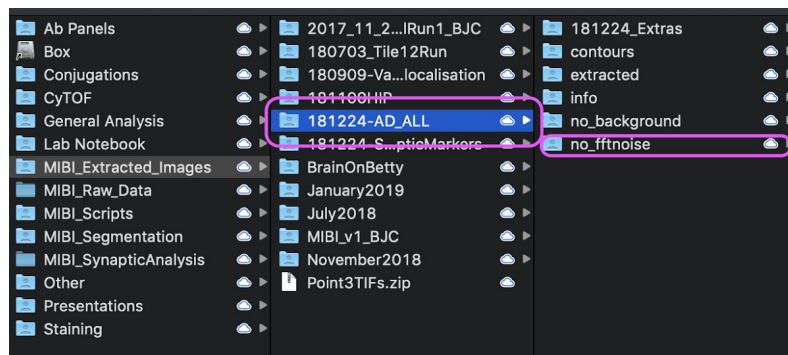
## EZ\_SEGMENTER WALKTHROUGH

General steps:

1. Add Points from MIBI run
2. Create Composite images on channels of interest
3. Create Mask using threshold, blur, and minimum pixel values
4. Create Objects & Save FCS

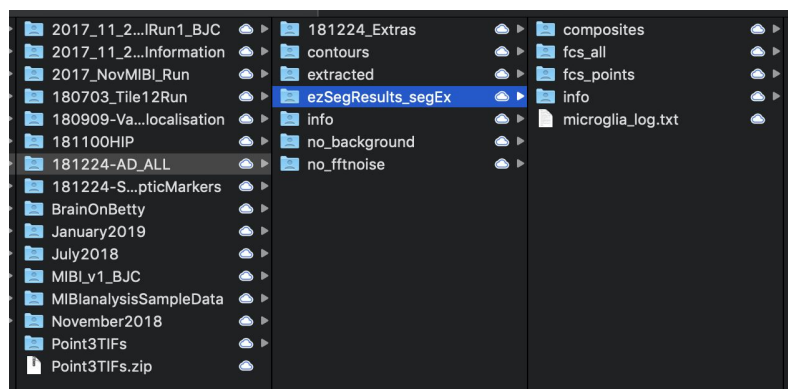
Start with: MIBI run folder (e.g. ~/RunA/)

- Points with **background removed, de-noised** tiff files. Also includes data.mat.
  - e.g. ~/RunA/Point#/data\_deNoised.mat
  - e.g. ~/RunA/Point#/TIFs/\*(all your images)
- Info folder (contains csv, run xml)
  - e.g. ~/RunA/info/panel.csv
  - e.g. ~/RunA/info/run.xml



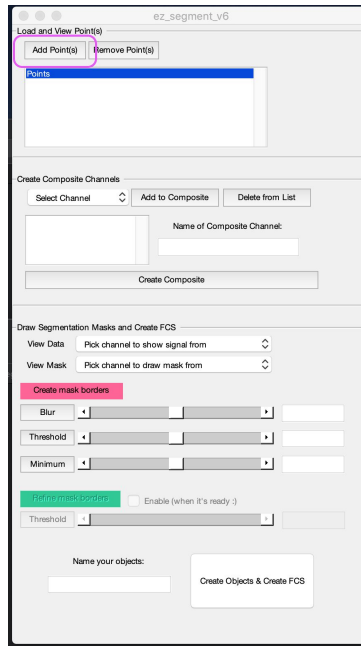
End with: MIBI run folder (e.g. ~/RunA/), segmentation run name (e.g. segEx)

- ~/RunA/ezSegResults\_segEx/composites/Point#/\*(composite tiffs)
- ~/RunA/ezSegResults\_segEx/fcs\_all/\*(fcs files all in one place)
- ~/RunA/ezSegResults\_segEx/fcs\_points/Point#/\*(fcs files per point)
- ~/RunA/ezSegResults\_segEx/info/\*(new csv)
- ~/RunA/ezSegResults\_segEx/log.txt

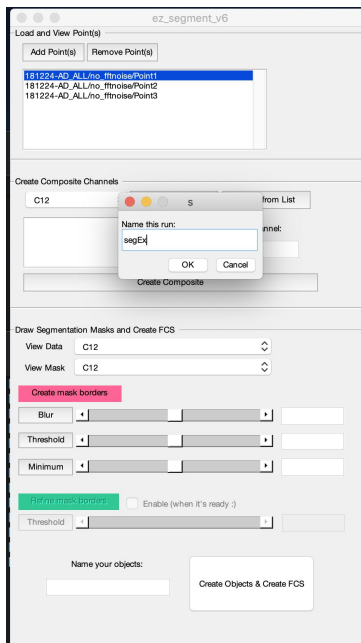


*Detailed steps:*

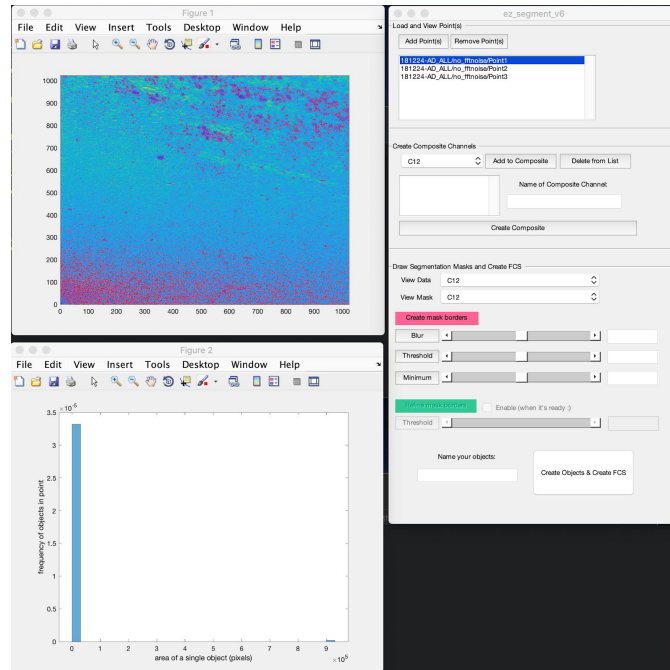
1. Add Points from MIBI run
  - a. Open up ez\_segementer\_gui in MIBI\_GUI (MATLAB) and click Run
  - b. Click on [Add Point(s)] and navigate file browser to MIBI run folder. Select points to add to the GUI.



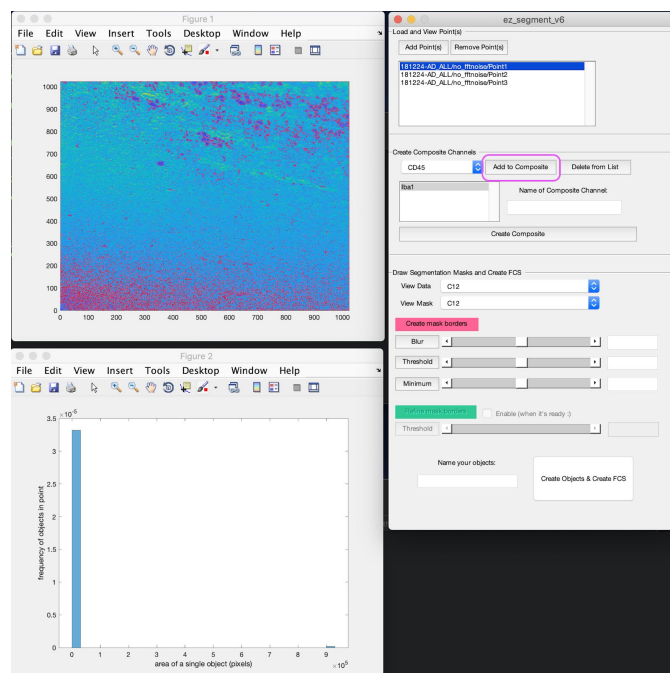
- c. Give your segmentation run a name (e.g. segEx)



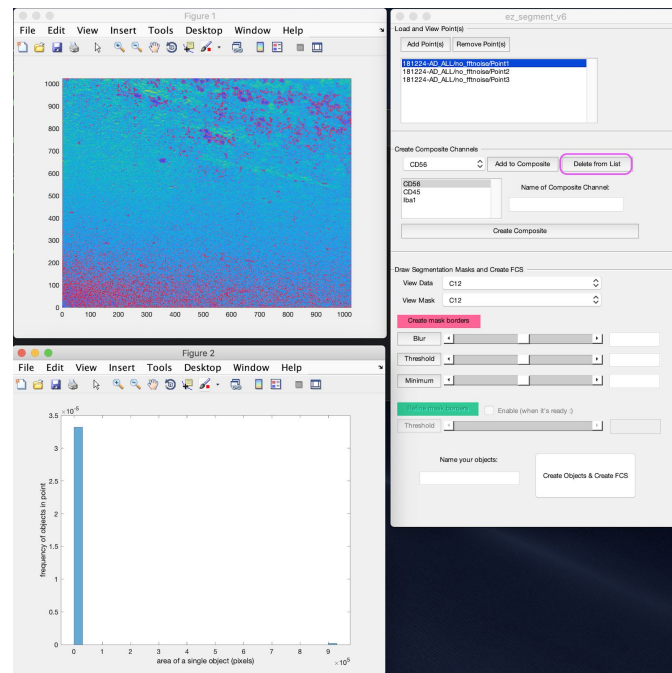
- d. Points will load into listbox, along with channels and count data. Two figures will additionally pop-up – one with an image of your currently selected data and mask channel, and another showing a histogram of object distribution. If these plots look off, don't worry, it's probably due to the fact the mask hasn't been optimized yet and the default channel is often Carbon.



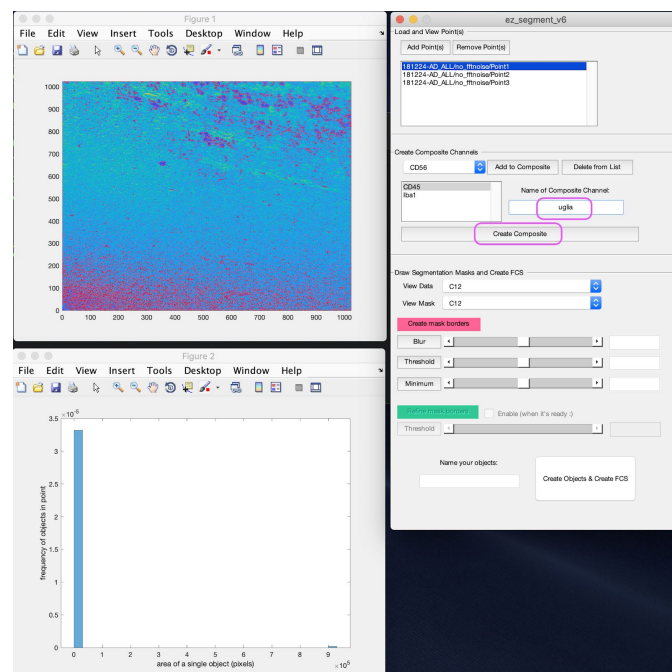
2. Create Composite images on channels of interest
  - a. If you want to segment on a composite of different channels (e.g. Iba1 and CD45 for microglia object identification), first add your individual channels of interest by selecting them from the drop down menu and selecting the [Add to Composite] button.



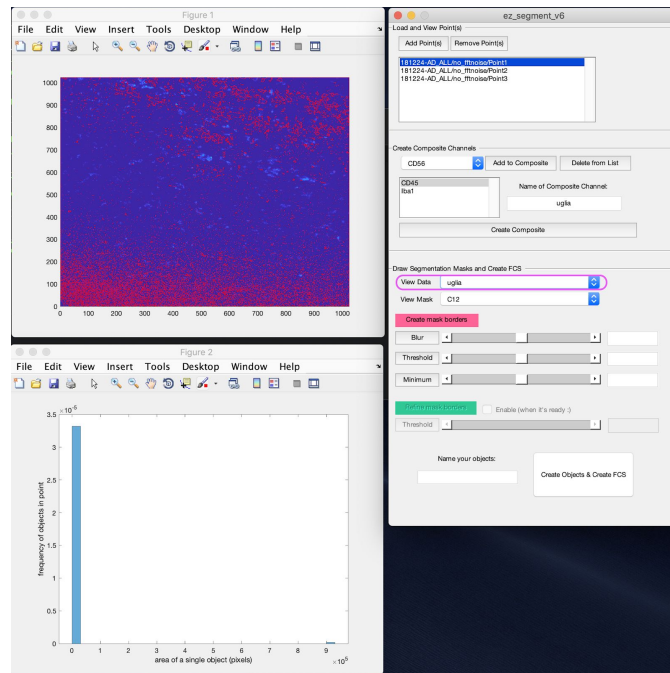
- b. If you want to remove a channel, select it in the listbox and select [Delete from List] button.
- c. When finished selecting channels, name your composite channel in the textbox (e.g. “uglia” for Iba1 and CD45).



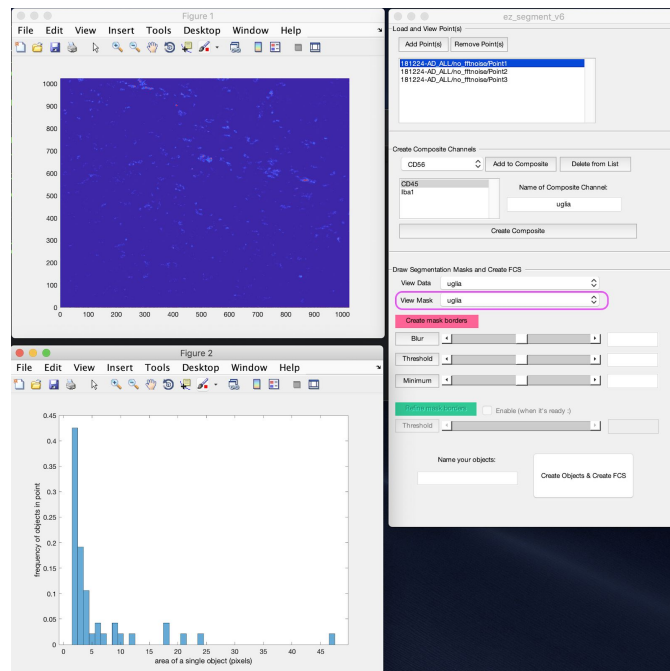
- d. Select [Create Composite]. When message appears, the composite tiff's have been saved and added into the composite folder, as well as added into the GUI's view and mask dropdown lists.



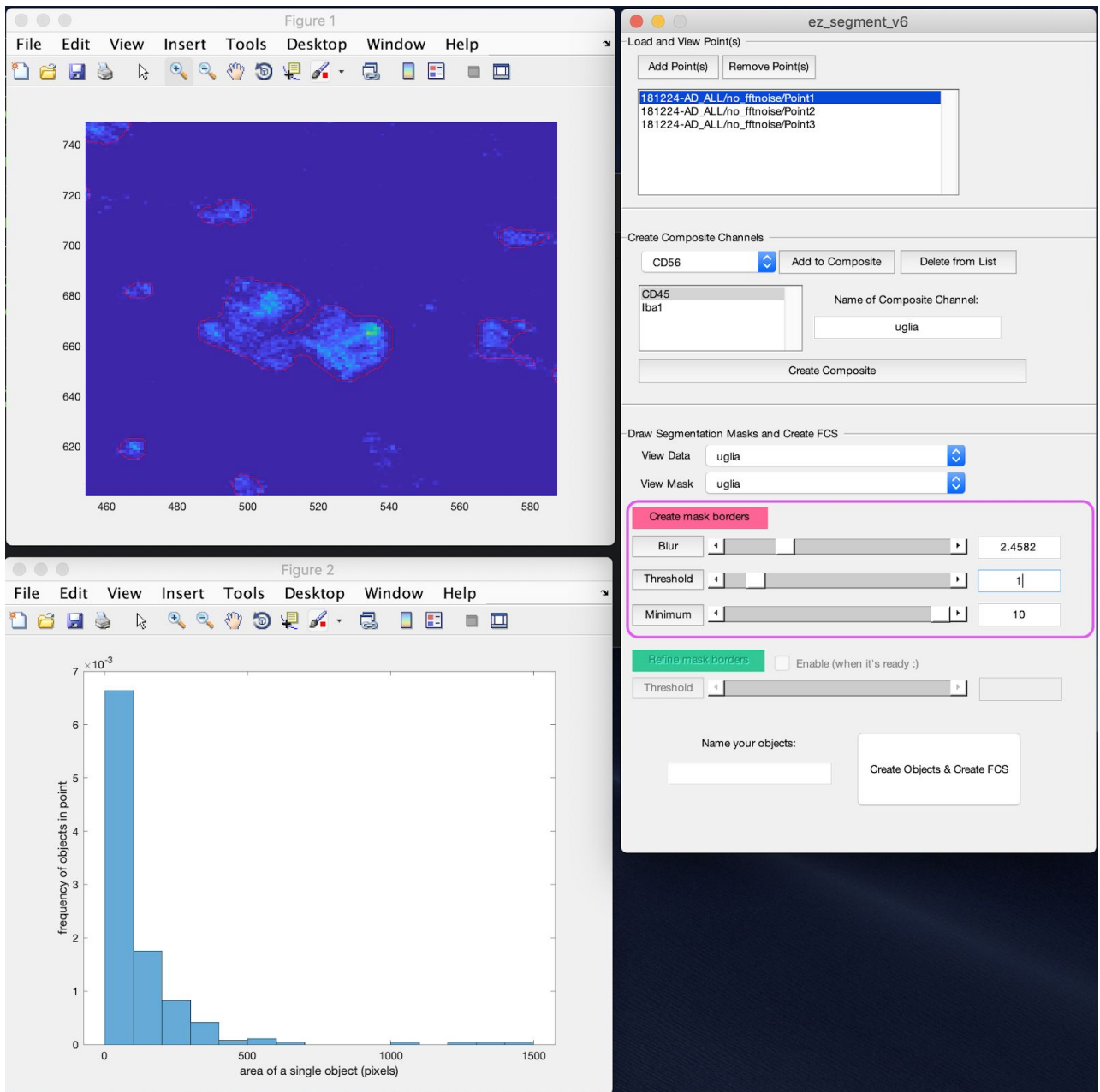
3. Create Mask using threshold, blur, and minimum pixel values
  - a. Select which channel you would like to view the image for in the [View Data] dropdown list (e.g. “uglia”). The image plot should update with signal distribution for that channel.



- b. Select which channel you would like to use to make a mask around [View Mask] dropdown list (e.g. microglia). The image plot should update with the signal distribution for that channel. The histogram should also update with a news distribution of objects identified with the default mask parameters.



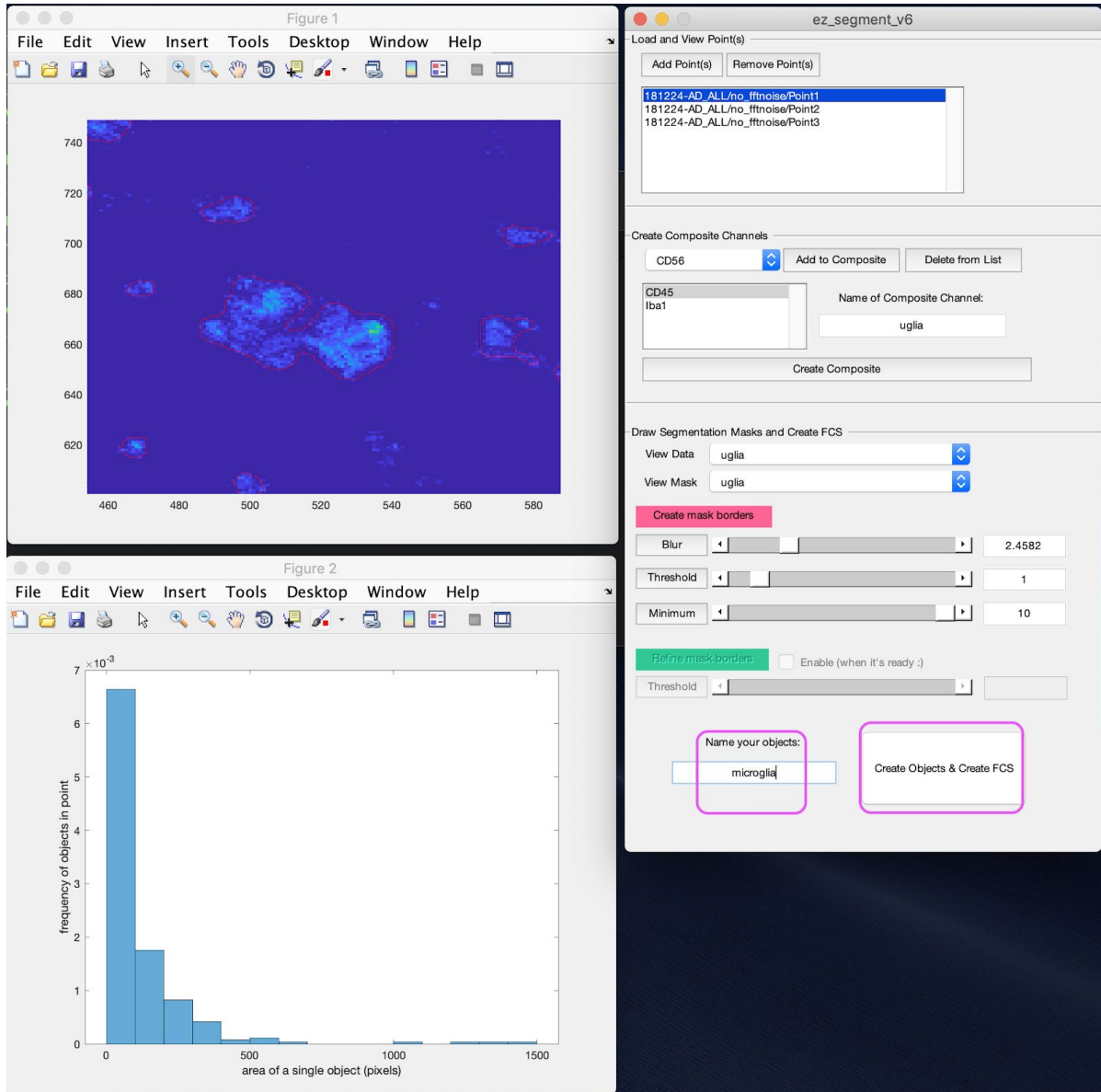
- c. Choose values to create the mask. Look at values, image plot, and histogram to determine best boundaries and object size to draw mask around.
  - i. Increasing blur will make mask borders smoother and more connected
  - ii. Increasing threshold will eliminate pixels with a signal lower than the increased value, masking around only those pixels that have signal intensity at or above the set value.
  - iii. Increasing minimum will eliminate objects that do not contain at least the given number of pixels.
  - iv. Can use buttons and text fields to change range or set exact values.
  - v. Once satisfied with mask boundaries, move onto next step.





#### 4. Create Objects & Save FCS

- In text box, name your objects that you have segmented out (e.g. “microglia”).
- Select [Create Objects & Create FCS]. If operation is successful, a popup notifying you of success will show up.



#### 5. EXTRA :::: Check your run

- A log.txt file will also show up in the segmentation folder, where you can look at time of segmentation, points segmented, mask values, and any composites made.

```
microglia_log.txt
time: 30-May-2019 14:35:34
points: 181224-AD_ALL/no_fftnoise/Point1, 181224-AD_ALL/no_fftnoise/Point2, 181224-AD_ALL/no_fftnoise/Point3,
composites: uqlia,
view_data: uqlia,
view_mask: uqlia,
named_objects: microglia,
blur: 2.4582,
threshold: 1,
minimum: 10
```